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Susceptibility of Peruvian Mosquitoes to Eastern Equine Encephalitis Virus

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ABSTRACT Mosquitoes were collected in the Amazon Basin, near Iquitos, Peru, and used in experimental studies to evaluate their susceptibility to strains of eastern equine encephalitis virus (EEEV) that were isolated from mosquitoes captured within 20 km of Iquitos. When fed on hamsters or chickens with a viremia of $\approx 10^5$ plaque-forming units (PFU) of EEEV/ml, *Culex pedroi* Sirivanakarn and Belkin, *Aedes fulvus* (Wiedemann), *Psorophora albigenu* (Peryassu), and *Psorophora ferox* (Von Humboldt) were susceptible to infection, whereas none of the *Aedes serratus* (Theobald), *Culex vomerifer* Komp, *Culex gnomatos* Sallum, Huchings, and Ferreira, *Culex portesi* Senevet and Abonnenc, or *Culex coronator* Dyar and Knab became infected, even though they fed on the same viremic blood sources. When these mosquito species fed on animals with viremias of $\approx 10^8$ PFU/ml, *Cx. pedroi*, *Ae. fulvus*, *Ps. albigenu*, and *Psorophora cingulata* (Fabricius) were the most susceptible. Mosquito species were susceptible to both a lineage II (Brazil-Peru) and a lineage III (Argentina-Panama) isolate of EEEV. This study, combined with the repeated isolation of strains of EEEV from *Cx. pedroi* captured in the Amazon Basin region of Peru, suggests that *Cx. pedroi* may be the primary enzootic vector of EEEV in this region.

KEY WORDS Peru, eastern equine encephalitis virus, transmission, mosquito

Eastern equine encephalitis virus (EEEV; family *Togaviridae*, genus *Alphavirus*) is enzootic in the eastern United States and throughout Central and South America, and infection with this virus in North America can lead to severe illness and death (Morris 1988). Based on genetic information, this virus can be separated into four subtypes (Weaver et al. 1994, Brault et al. 1999). These consist of lineage I, found in North America, and three found in South America: lineage II (Brazil-Peru; isolates found in Brazil, Guatemala, and Peru), lineage III (Argentina-Panama; isolates found in Argentina, Brazil, Colombia, Ecuador, Guiana, Panama, Peru, Trinidad, and Venezuela), and lineage IV (based on a single isolate from Brazil) (Brault et al. 1999). Although the transmission cycle for this virus in North America is well described, with *Culiseta melanura* (Coquillett) being the primary enzootic vector and various passerine birds serving as amplifying hosts, little is known about the epidemiology of this virus in Central and South America.

As part of a field ecology study conducted in the Amazon Basin region in Peru, mosquitoes were captured and identified and tested for arboviruses (Pecor et al. 2000, Jones et al. 2004, Turell et al. 2005). A total of 166 viral isolates were obtained from these mosquitoes, including 39 isolates of EEEV, and nearly all of these EEEV isolations were associated with *Culex* (*Melanoconion*) *pedroi* Sirivanakarn and Belkin (Turell et al. 2005). Genetic analysis of these isolates indicated that viruses in both lineages II and III were co-circulating in the Amazon Basin region of Peru (Turell et al. 2005, Kondig et al. 2007).

Studies on the ability of mosquitoes to transmit these South American strains of EEEV have not been conducted. Therefore, we evaluated the vector competence of several Peruvian mosquito species collected in an area where both lineage II and lineage III strains of EEEV were isolated from mosquitoes. Field-collected mosquitoes were allowed to feed on EEEV-infected adult hamsters (*Mesocricetus auratus*) or young chickens (*Gallus gallus*), and rates of infection,

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14. ABSTRACT

Mosquitoes were collected in the Amazon Basin, near Iquitos, Peru, and used in experimental studies to evaluate their susceptibility to strains of eastern equine encephalitis virus (EEEV) that were isolated from mosquitoes captured within 20 Km of Iquitos. When fed on hamsters or chickens with a viremia of ~105 plaque-forming units (PFU) of virus per ml, Culex pedroi Sirivanakarn and Belkin, Aedes fulvus (Wiedemann), Psorophora albigenu (Peryassu), and Psorophora ferox (Von Humboldt) were susceptible to infection, while none of the Aedes serratus (Theobald), Culex vomerifer Komp, Culex gnomatos Sallum, Huchings, & Ferreira, Culex portesi Senevet and Abonnenc, or Culex coronator Dyar & Knab became infected, even though they fed on the same viremic blood sources. When these mosquito species fed on animals with viremias ~108 PFU per ml, Cx. pedroi, Ae. fulvus, Ps. albigenu, as well as Psorophora cingulata (Fabricius), were the most susceptible. Mosquito species were susceptible to both a Lineage II (Brazil-Peru) as well as a Lineage III (Argentina-Panama) isolate of EEEV. This study, combined with the repeated isolation of strains of EEEV from Cx. pedroi captured in the Amazon Basin region of Peru, indicate that Cx. pedroi is the primary enzootic vector of EEEV in this region.

15. SUBJECT TERMS

eastern equine encephalitis virus, EEE, mosquito vector, Peru, susceptiblity, transmission

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dissemination, and transmission were determined for individual mosquito species.

Materials and Methods

Mosquitoes. Adult female mosquitoes were collected in dry ice-baited CDC miniature light traps or as they landed on humans near the rural village of Puerto Almendra, located in a forested area in the Amazon Basin near Iquitos, Peru (3°07′ S, 73°3′ W), from April 1996 through August 1998. Both lineage II and lineage III strains of EEEV were circulating in the area where these mosquitoes were captured (Turell et al. 2005). Mosquitoes were transported to a biosafety level (BSL)-3 (with HEPA-filtered exhaust air, treated sewage, and a 100% clothing change) laboratory at the U.S. Army Medical Research Institute of Infectious Diseases, Fort Detrick, MD; provided apple slices as a carbohydrate source; and held at 26°C for 1–3 d until exposed to EEEV. Species studied included *Aedes fulvus* (Wiedemann), *Aedes serratus* (Theobald), *Culex* (*Melanoconion*) *gnomatos* Sallum, Huchings, and Ferreira, *Cx. pedroi*, *Culex* (*Melanoconion*) *portesi* Senevet and Abonnenc, *Culex* (*Melanoconion*) *vomerifer* Komp, *Culex* (*Culex*) *coronator* Dyar and Knab, *Culex* (*Culex*) *declarator/mollis* [a mixture of *Culex* (*Culex*) *declarator* Dyar and Knab and *Culex* (*Culex*) *mollis* Dyar and Knab], *Psorophora albigenu* (Peryassu), *Psorophora cingulata* (Fabricius), and *Psorophora ferox* (Von Humboldt). Voucher specimens were placed in the collection at the National Museum of Natural History, Smithsonian Institution, Washington, DC. Research was conducted in compliance with the Animal Welfare Act and other federal statutes and regulations relating to animals and experiments involving animals and adheres to principles stated in the Guide for the Care and Use of Laboratory Animals, National Research Council, 1996. The facility where this research was conducted is fully accredited by the Association for Assessment and Accreditation of Laboratory Animal Care International.

Virus and Virus Assay. We used both a lineage II (PE-3.0815) and a lineage III (PE-0.0155) strain of EEEV. Both of these strains had been isolated from *Cx. pedroi* captured in a forested area near Puerto Almendra in 1996 and had been passaged twice in Vero cells before use in this study (Turell et al. 2005). Sequencing of these two viruses indicated that there was 81.8% identity for the entire genome, with 82.1 and 67.5% identity in the *E3* and *nsP3* genes, respectively (Kondig et al. 2007).

Serial 10-fold dilutions of specimens were tested for infectious virus by plaque assay on Vero cell monolayers as described by Gargan et al. (1983) except that the neutral red stain was added 2, rather than 4, d after applying the initial agarose overlay.

Viremia Profile Studies. Preliminary studies were conducted to determine viremia profiles for EEEV in young (1–13 d old) leghorn chickens or adult female (>90 g) Syrian hamsters. Young chickens were inoculated subcutaneously with 0.1 ml of a suspension containing $\approx 10^4$ plaque-forming units (PFU) of EEEV. These chickens were bled daily from the jugular vein (0.1 ml of blood into 0.9 ml of heparinized diluent, 10% fetal bovine serum in medium 199 with Earle's salts and antibiotics). Similarly, hamsters were inoculated intraperitoneally with 0.2 ml of a suspension containing 10^4 PFU of EEEV and bled daily by cardiac puncture. Blood samples were frozen at −70°C until tested for virus by plaque assay in Vero cells.

Determination of Vector Competence. Adult female Syrian hamsters (>90 g) and young leghorn chickens (1–5 d old) were infected as described above to serve as a viremic blood meal for the mosquitoes. These animals were either anesthetized (hamsters) or restrained (chickens) 1 or 2 d after infection and placed on top of 3.8-liter screen-topped cardboard cages that contained field-collected mosquitoes for 45 min. Immediately after each mosquito feeding, the infected animals were bled as described above and the blood suspensions stored at −70°C until tested for virus by plaque assay to determine the viremia at the time of mosquito feeding.

Engorged mosquitoes were transferred to a 3.8-liter screen-topped cardboard cage and nonengorged mosquitoes were killed and discarded or inoculated intrathoracically (Rosen and Gubler 1974) with 0.3 μl of a suspension containing $\approx 10^{1.2}$ PFU ($10^{4.7}$ PFU/ml) of virus to determine transmission rates for individual mosquitoes with a disseminated viral infection. An apple slice, or a 10% sucrose solution, was provided as a carbohydrate source, and mosquitoes were held at 26°C at a 16:8 (L:D) h photoperiod. After an extrinsic incubation period of 12–22 d (>80% tested 13–17 d), mosquitoes were allowed to feed on susceptible 1- to 2-d-old chickens either individually or in small groups of two to five mosquitoes of the same species to determine transmission rates. Immediately after each transmission trial, mosquitoes were killed by freezing at −20°C for 2–5 min, identified to species, their legs and bodies triturated separately in 1 ml of diluent, and suspensions frozen at −70°C until tested for virus by plaque assay. Chickens fed on during the transmission attempt were bled 24 h later as described above and the blood was frozen at −70°C until tested for EEEV by plaque assay. Because all viremias detected in 1- to 2-d-old chickens were $>10^8$ PFU/ml, it is unlikely to have missed a virus-infected chicken.

Estimates of infection and dissemination rates were determined by assaying individual mosquito body tissue and leg suspensions for virus. If virus was recovered from its body, but not its legs, the mosquito was considered to have a nondisseminated infection limited to its midgut. Alternatively, if virus was recovered both from body and leg suspensions, the mosquito was considered to have a disseminated infection (Turell et al. 1984). The infection rate was defined as the percentage of all mosquitoes tested containing virus in the body sample, and the dissemination rate was defined as the percentage of all mosquitoes tested (regardless of infections status) containing virus in the legs sample. We used the extended Wald method for calculating 95% CIs (Agresti and Coull 1998).

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Table 1. Viremias in hamsters and young chickens after inoculation of about 10^4 PFU of EEEV

Species	Age at infection (d)	Virus	Lineage	Days after infection		
				1	2	3
Chicken	1	PE-0.0155	III	8.8 (±0.2) (4)[a]	8.9 (1)	Dead
Chicken	9	PE-0.0155	III	6.3 (±0.2) (2)	<3 (2)	<3 (2)
Chicken	8	PE-3.0815	II	NT	<3 (1)	NT
Chicken	13	PE-3.0815	II	8.2 (±0.8) (2)	NT	NT
Hamster	Adult	PE-0.0155	III	5.3 (±2.0) (6)	5.4 (±0.3) (4)	≤3.5 (±2.0) (2)
Hamster	Adult	PE-3.0815	II	4.3 (±0.6) (3)	4.6 (±0.8) (4)	<2.0 (2)

[a] Mean logarithm$_{10}$ PFU/ml of blood (SD) (no. tested).
NT, not tested.

Results

Viremias in Vertebrates. Peak viremias in hamsters and chickens infected with the two strains of EEEV ranged from $10^{4.6}$ to $10^{8.9}$ PFU/ml (Table 1), with peak viremias being significantly ($T = 4.92$, $df = 11$, $P < 0.001$) higher in 1-d-old chickens than in adult hamsters. However, viremias in chickens that were 8–13 d old when inoculated were significantly lower ($T = 2.53$, $df = 6$, $P = 0.045$) than in chickens that were 1 d old when inoculated. In addition, chickens inoculated when ≥8 d old all survived, whereas all those inoculated when 1 d old died or were killed when moribund at <48 h after infection. Testing of blood immediately after mosquito feeding indicated that mosquitoes were exposed to two dose ranges: one ≈10^5 (range, $10^{4.6}$–$10^{5.8}$) and the other ≈$10^{8.2}$ (range, $10^{7.7}$–$10^{8.5}$) PFU/ml, respectively.

Susceptibility of Mosquitoes to Oral Infection with EEEV. Mosquitoes ingesting the two lineages of EEEV had similar infection patterns (Table 2). After feeding on hamsters or chickens with a viremia of ≈10^5 PFU of virus/ml, *Cx. pedroi*, *Ae. fulvus*, *Ps. albigenu*, and *Ps. ferox* were all susceptible to infection with one or both lineages, whereas none of the *Ae. serratus*, *Cx. vomerifer*, *Cx. gnomatos*, *Cx. portesi*, or *Cx. coronator* became infected. When fed on animals with viremias ≈10^8 PFU/ml, all of the mosquito species tested were susceptible to a lineage III strain of EEEV (lineage II not tested); however, *Cx. pedroi*, *Ae. fulvus*, *Ps. albigenu*, and *Ps. cingulata* were more susceptible to infection than the other species tested. Although most mosquito species were not tested against both lineages at the same dose, we did not observe any consistent differences in susceptibility to either a lineage II or a lineage III isolate of EEEV.

Viral Dissemination and Transmission. After feeding on hamsters or chickens with a viremia of ≈10^5 PFU of virus/ml, only *Cx. pedroi*, *Ae. fulvus*, and *Ps. albigenu* developed a disseminated infection. When mosquitoes ingested a viremia ≈10^8 PFU/ml, at least 50% of *Cx. pedroi*, *Ae. fulvus*, *Ps. albigenu*, and *Ps. cingulata* developed a disseminated infection. In con-

Table 2. Susceptibility of Peruvian mosquitoes to EEEV after feeding on viremic hamsters or young chickens

Species	PE-3.0815 (lineage II)			PE-0.0155 (lineage III)			Combined		
	No. tested	Infection rate[a]	Dissem. rate[b]	No. tested	Infection rate[a]	Dissem. rate[b]	No. tested	Infection rate[a]	Dissem. rate[b]
Infectious dose = $10^{4.6}$–$10^{5.8}$ PFU/ml									
Culex (*Mel.*) *pedroi*		NT		18	72	17	18	72 (49–88)	17 (5–40)
Aedes (*Och.*) *fulvus*	3	100	0	6	50	33	9	67 (35–88)	22 (5–56)
Psorophora (*Jan.*) *albigenu*	13	85	62	4	0	0	17	60 (36–80)	47 (25–70)
Psorophora (*Jan.*) *ferox*	2	50	0		NT		2	50 (9–99)	0 (0–71)
Aedes (*Och.*) *serratus*	37	0	0	18	0	0	55	0 (0–8)	0 (0–8)
Culex (*Mel.*) *gnomatos*	4	0	0	1	0	0	5	0 (0–49)	0 (0–49)
Culex (*Mel.*) *portesi*		NT		6	0	0	6	0 (0–44)	0 (0–44)
Culex (*Mel.*) *vomerifer*	1	0	0	12	0	0	13	0 (0–27)	0 (0–27)
Culex (*Cux.*) *coronator*	5	0	0	41	0	0	46	0 (0–9)	0 (0–9)
Infectious dose = $10^{7.7}$–$10^{8.5}$ PFU/ml									
Culex (*Mel.*) *gnomatos*		NT		2	100	0	2	100 (29–100)	0 (0–71)
Aedes (*Och.*) *fulvus*		NT		14	100	50	14	100 (75–100)	50 (27–73)
Culex (*Mel.*) *pedroi*		NT		7	86	57	7	86 (47–99)	57 (25–84)
Psorophora (*Gra.*) *cingulata*		NT		4	75	50	4	75 (29–97)	50 (15–85)
Psorophora (*Jan.*) *albigenu*		NT		4	75	50	4	75 (29–97)	50 (15–85)
Aedes (*Och.*) *serratus*		NT		15	47	13	15	47 (25–70)	13 (2–39)
Psorophora (*Jan.*) *ferox*		NT		14	43	21	14	43 (7–48)	21 (7–48)
Culex (*Mel.*) *portesi*		NT		26	19	8	26	19 (8–38)	8 (1–25)
Culex (*Cux.*) *coronator*		NT		16	6	0	16	6 (1–30)	0 (0–23)
Culex (*Cux.*) *declorator/mollis*[c]		NT		7	0	0	7	0 (0–40)	0 (0–40)

[a] Percentage of mosquitoes containing virus in their bodies (95% CI).
[b] Percentage of all mosquitoes tested containing virus in their legs (95% CI).
[c] Consisted of *Cx. declarator* and *Cx. mollis*.
NT, not tested.

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Table 3. Transmission rates for mosquitoes with a disseminated infection with EEEV after either oral exposure or intrathoracic inoculation

Species	Route of infection				Totals	
	Oral[a]		Inoculated			
	No. tested[b]	Transmission rate[c]	No. tested[b]	Transmission rate[c]	No. tested[b]	Transmission rate[c]
Ochlerotatus (*Och.*) *fulvus*	2	0	3[d]	0	5[d]	0
Culex (*Mel.*) *pedroi*		NT	1	100	1	100
Psorophora (*Gra.*) *cingulata*	1	0	1	0	2	0
Psorophora (*Jan.*) *albigenu*	2	0	1	100	3	33
Aedes (*Och.*) *serratus*	1	0	8	13	9	11
Psorophora (*Jan.*) *ferox*		NT	10	40	10	40

[a] Mosquitoes with a disseminated infection (virus in their legs) after oral exposure to EEEV.
[b] Number of mosquitoes that fed.
[c] Percentage of mosquitoes that fed that transmitted virus.
[d] In addition, two pair of mosquitoes with a disseminated infection fed and did not transmit virus. Because they fed in a pool, they were not included in the transmission data; however, as neither pool transmitted virus, none of nine with a disseminated infection transmitted EEEV.
NT, not tested.

trast, the highest dissemination rate in any of the other species tested was 21%.

Unfortunately, only a few mosquitoes with a disseminated infection fed on naïve 1- to 2-d-old chickens to determine transmission and thus we have only limited data pertaining to the mosquito-host portion of the transmission cycle. Despite being among the most susceptible to infection and dissemination with EEEV, *Ae. fulvus* did not transmit EEEV by bite, even though nine individuals with a disseminated infection fed on susceptible chickens (Table 3). In contrast, the only *Cx. pedroi* with a disseminated infection tested did transmit EEEV by bite.

Discussion

This is the first reported study on the vector competence of South American mosquito species for South American strains of EEEV. *Culex pedroi*, *Ae. fulvus*, and the three *Psorophora* spp. tested seemed to be the most susceptible to infection. However, the failure of any of nine *Ae. fulvus* with a disseminated infection to transmit virus by bite when fed on young chickens indicated that this species has a significant salivary gland barrier (Kramer et al. 1981, Hardy 1988) and probably does not have a significant role in the natural transmission and maintenance cycle of South American strains of EEEV. This pattern of high susceptibility of *Ae. fulvus* to infection and dissemination with an *Alphavirus*, yet having a major salivary gland barrier, was also observed when this species was exposed to various viruses in the Venezuelan equine encephalitis virus (VEEV; family *Togaviridae*, genus *Alphavirus*) complex and may indicate that, whereas highly susceptible to alphaviruses, it should not be considered an important natural vector (Turell et al. 2000, 2006). In contrast, *Cx. pedroi* was highly susceptible to infection, even when they were exposed to a low viremia, and the only specimen with a disseminated infection that refed transmitted EEEV by bite. Although highly susceptible to EEEV, *Cx. pedroi* was a relatively inefficient vector of various subtype I and III viruses in the VEEV complex (Turell et al. 2000, 2006). Although it is possible that the use of strains of EEEV isolated from *Cx. pedroi* mosquitoes may have been preselected for ones that would replicate in this species, the fact that nearly all (34/37) of the EEEV isolates obtained from identified species in a previous study were from *Cx. pedroi* indicate that this species is likely to be involved in nature (Turell et al. 2005).

As reported for several members of the VEEV complex (Turell et al. 2000, 2006), *Cx. coronator* was virtually refractory to infection with EEEV. Results for the combined *Cx. declarator/mollis* were similar in that none of nine mosquitoes that fed on a hamster with a high viremia became infected. Although sample sizes were small, the other *Cx.* (*Mel.*) spp. tested (*Cx. vomerifer*, *Cx. gnomatos*, and *Cx. portesi*) all seemed to be less susceptible to EEEV infection than was *Cx. pedroi.* In contrast, previous studies showed that *Cx. gnomatos* was significantly more susceptible than *Cx. pedroi* to infection with a Mucambo-like, subtype IIIC virus in the VEEV complex (Turell et al. 2006). All of the *Psorophora* spp. tested seemed to be moderately competent, and both species, *Ps. albigenu* and *Ps. ferox*, for which at least three mosquitoes with a disseminated infection fed on a susceptible chicken, transmitted virus by bite. Because of the periodic large numbers of these flood-water mosquitoes, the isolation of EEEV from *Ps. albigenu* caught in this region (Turell et al. 2005) and their avidity to bite humans and other large mammals, these species may serve as bridge vectors from the enzootic *Cx.* (*Mel.*) spp.–small mammal cycle to humans.

Little is known about potential vertebrate amplifying hosts of EEEV in South and Central America. EEEV antibodies have been detected in both rodent and avian species (Monath et al. 1985), but the mere presence of antibodies does not mean that the vertebrate host was capable of producing a viremia of sufficient magnitude to infect mosquitoes. Although both lineage II and III EEEV produced viremias in both avian and rodent models in these studies of sufficient magnitude to infect potential vectors, the species used in this study are laboratory models and do not represent the actual species involved in the natural trans-

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mission cycle in Central and South America. Studies are, therefore, needed to evaluate avian and rodent species from areas where these viruses are enzootic.

Vector competence is only one aspect in the determination of vectorial capacity. Mosquito density, biting behavior, longevity, and seasonal activity must all be taken into account in determining the potential importance of a vector. Although we did not conduct bloodmeal identification studies, *Cx. pedroi* is highly attracted to humans, with human landing collection rates of 32.8 captured per 24-h period per person, and >99% of these were captured during hours of darkness (Jones et al. 2004). In addition, of the 39 isolations of EEEV made from mosquitoes captured in the same study area reported by Turell et al. (2006), 34 were made from *Cx. pedroi* and two others were from *Cx.* (*Mel.*) spp. containing a mix of *Cx. pedroi* and other *Cx.* (*Mel.*) spp. Also, EEEV was detected in six pools of *Cx. pedroi* captured in this area in a separate study (O'Guinn et al. 2004). Thus, because *Cx. pedroi* is an efficient laboratory vector of EEEV, among the more common mosquitoes collected, and are naturally infected, they should be considered the principle vector of EEEV virus in the Amazon Basin region of Peru. In addition, because they readily land on and attempt to bite humans and were frequently collected in human landing collections in a nearby village (unpublished data), they probably also serve as a bridge vector between the enzootic cycle and human infections in this region. However, in other regions in South and Central America, EEEV has been isolated from other species in the subgenus *Cx.* (*Mel.*), including *Culex* (*Mel.*) *panocossa* Dyar, *Culex* (*Mel.*) *dunni* Dyar, and *Culex* (*Mel.*) *taeniopus* Dyar and Knab (Srihongse and Galindo 1967, Walder et al. 1984). The repeated isolation of EEEV from species within this subgenus throughout South and Central America indicates the importance of the role of members of this subgenus in the epidemiology of EEEV.

Additional studies are needed to clarify the host–vector relationships and to define the enzootic maintenance cycle. These should include studies on mosquitoes, including host preference, time of day and season of biting activity, population density, etc. Studies are needed on vertebrate competence in appropriate mammalian and avian species for South American strains of EEEV and to compare the vector competence of mosquitoes for South and North American strains of EEEV. These data are needed to understand the natural transmission cycle(s) of EEEV in South and Central America.

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References Cited

Agresti, A., and B. A. Coull. 1998. Approximate is better than "Exact" for interval estimation of binomial proportions. Am. Stat. 52: 119–126.

Brault, A. C., A. M. Powers, C. L. Chavez, R. N. Lopez, M. F. Cachon, L. F. Gutierrez, W. Kang, R. B. Tesh, R. E. Shope, and S. C. Weaver. 1999. Genetic and antigenic diversity among eastern equine encephalitis viruses from North, Central, and South America. Am. J. Trop. Med. Hyg. 61: 579–586.

Gargan, T. P., II, C. L. Bailey, G. A. Higbee, A. Gad, and S. El Said. 1983. The effect of laboratory colonization on the vector pathogen interactions of Egyptian *Culex pipiens* and Rift Valley fever virus. Am. J. Trop. Med. Hyg. 32: 1154–1163.

Hardy, J. L. 1988. Susceptibility and resistance of vector mosquitoes, pp. 87–126. *In* T. P. Monath (ed.), The arboviruses: epidemiology and ecology, vol. 1. CRC, Boca Raton, FL.

Jones, J. W., M. J. Turell, M. R. Sardelis, D. M. Watts, R. E. Coleman, R. Fernandez, F. Carbajal, J. E. Pecor, C. Calampa, and T. A. Klein. 2004. Seasonal distribution, biology, and human attraction patterns of culicine mosquitoes (Diptera: Culicidae) in a forest near Puerto Almendras, Iquitos, Peru. J. Med. Entomol. 41: 349–360.

Kondig, J. P., M. J. Turell, J. S. Lee, M. L. O'Guinn, and L. P. Wasieloski, Jr. 2007. Genetic analysis of South American eastern equine encephalomyelitis viruses isolated from mosquitoes collected in the Amazon Basin region of Peru. Am. J. Trop. Med. Hyg. 76: 408–416.

Kramer, L. D., J. L. Hardy, S. B. Presser, and E. J. Houk. 1981. Dissemination barriers for western equine encephalomyelitis virus in *Culex tarsalis* infected after ingestion of low viral doses. Am. J. Trop. Med. Hyg. 30: 190–197.

Monath, T. P., M. S. Sabattini, R. Pauli, J. F. Daffner, C. J. Mitchell, G. S. Bowen, and C. B. Cropp. 1985. Arbovirus investigations in Argentina, 1977–1980. IV. Serologic surveys and sentinel equine program. Am. J. Trop. Med. Hyg. 34: 966–975.

Morris, C. D. 1988. Eastern equine encephalitis, pp. 1–20. *In* T. P. Monath (ed.), The arboviruses: epidemiology and ecology, vol. III. CRC, Boca Raton, FL.

O'Guinn, M. L., J. S. Lee, J. P. Kondig, R. Fernandez, and F. Carbajal. 2004. Field detection of eastern equine encephalitis virus in the Amazon Basin region of Peru using reverse transcription-polymerase chain reaction adapted for field identification of arthropod-borne pathogens. Am. J. Trop. Med. Hyg. 70: 164–171.

Pecor, J. E., J. Jones, M. J. Turell, R. Fernandez, F. Carbajal, M. O'Guinn, M. Sardelis, D. Watts, M. Zyzak, C. Calampa, and T. A. Klein. 2000. Annotated checklist of the mosquito species encountered during arboviral studies in Iquitos, Peru (Diptera: Culicidae). J. Am. Mosq. Control Assoc. 16: 210–218.

Rosen, L., and D. Gubler. 1974. The use of mosquitoes to detect and propagate dengue viruses. Am. J. Trop. Med. Hyg. 23: 1153–1160.

Srihongse, S., and P. Galindo. 1967. The isolation of eastern equine encephalitis virus from *Culex* (*Melanoconion*) *taeniopus* Dyar and Knab in Panama. Mosq. News 27: 74–76.

Turell, M. J., T. P. Gargan, I. I., and C. L. Bailey. 1984. Replication and dissemination of Rift Valley fever virus in *Culex pipiens.* Am. J. Trop. Med. Hyg. 33:176–181.

Approved for public release. Distribution is unlimited.

Turell, M. J., J. W. Jones, M. R. Sardelis, D. J. Dohm, R. E. Coleman, D. M. Watts, R. Fernandez, C. Calampa, and T. A. Klein. 2000. Vector Competence of Peruvian mosquitoes (Diptera: Culicidae) for epizootic and enzootic strains of Venezuelan equine encephalomyelitis virus. J. Med. Entomol. 37: 835–839.

Turell, M. J., M. L. O'Guinn, J. W. Jones, M. R. Sardelis, D. J. Dohm, D. M. Watts, R. Fernandez, A. Travassos Da Rosa, H. Guzman, R. Tesh, C. A. Rossi, G. V. Ludwig, J. A. Mangiafico, J. Kondig, L. P. Wasieloski, Jr., J. Pecor, M. Zyzak, G. Schoeler, C. N. Mores, C. Calampa, J. Lee, and T. A. Klein. 2005. Isolation of viruses from mosquitoes collected in the Amazon Basin region of Peru. J. Med. Entomol. 42: 891–898.

Turell, M. J., D. J. Dohm, R. Fernandez, C. Calampa, and M. L. O'Guinn. 2006. Vector competence of Peruvian mosquitoes (Diptera: Culicidae) for a subtype IIIC virus in the Venezuelan equine encephalomyelitis complex isolated from mosquitoes captured in Peru. J. Am. Mosq. Control Assoc. 22: 70–75.

Walder, R., O. M. Suarez, and C. H. Calisher. 1984. Arbovirus studies in the Guajira region of Venezuela: activities of eastern equine encephalitis and Venezuelan equine encephalitis viruses during an interepizootic period. Am. J. Trop. Med. Hyg. 33: 699–707.

Weaver, S. C., A. Hagenbaugh, L. A. Bellew, L. Gousset, V. Mallampalli, J. J. Holland, and T. W. Scott. 1994. Evolution of alphaviruses in the eastern equine encephalomyelitis complex. J. Virol. 68: 158–169.

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